20 years of gene therapy for SCID

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Severe combined immunodeficiency conditions are devastating disorders of adaptive immunity. Although these diseases were initially treated by transplantation of allogeneic hematopoietic stem cells, the past 20 years has shown that these conditions are correctable by gene therapy.

More than 50 years ago, the term 'severe combined immunodeficiency' (SCID) was coined to designate rare, lethal conditions in which infants die from an array of infections associated with a lack of lymphocytes in the blood. It was subsequently recognized that SCID covers a variety of genetic defects, all of which lead to profoundly impaired differentiation of T lymphocytes and (in some cases) additional blocks in the differentiation of B lymphocytes and/or natural killer (NK) lymphocytes. The molecular analysis of these conditions generated a flood of information on key steps in lymphocyte development[1]. Meanwhile, the term 'SCID' became very popular in the immunological community, as natural SCID mouse mutants were discovered and were widely used for the study of lymphocyte ontogeny and to serve as recipients for allogeneic and, above all, xenogeneic (human) grafts. Once the human leukocyte antigen (HLA) system and its involvement in allogeneic responses were progressively deciphered in the 1950s and 1960s, it became conceivable to cure babies with SCID by transplantation of HLA-identical bone marrow cells. Given the absence of T cells in these patients, it was thought that SCID sufferers could not reject a graft. This is what Good reported in 1968 (ref. 2), which paved the way to the development of transplantation of allogeneic hematopoietic stem cells (HSCs) for a much wider range of conditions. Remarkably, the success of transplantation of HLA-identical bone marrow into patients with SCID did not require the destruction of host stem cells. In the absence of an HLA-identical donor, transplantation of allogeneic HSCs, however, is still marred by the devastating consequences of graft-versus-host disease. This situation is what prompted the emergence of the concept of gene therapy[3], together with the known properties of retroviruses[4]. Again, SCID seemed to be the best condition in which to test the feasibility of this new approach, which led to the first clinical trial 20 years ago.

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Initial attempts to use gene therapy in adenosine deaminase deficiency

Deficiency in adenosine deaminase (ADA) was the first SCID condition for which a genetic and molecular cause was identified[1]. The absence of ADA leads to the accumulation of (de)adenosine compounds that induce cell death (mostly of lymphoid progenitors) and thus a nearly total absence of lymphocytes (**Fig. 1**). Given that ADA is ubiquitously expressed, this deficiency also induces other abnormalities. Indeed, ADA deficiency was considered a metabolic disease. Hence, enzyme-replacement therapy (ERT) with repeated injections of pegylated, purified ADA was suggested[5]. This therapy led to substantial improvements in lymphopoiesis and survival, although the patients remained dependent on supplementation and the disease correction was only partial.

This state is where gene therapy entered the scene, following the basic concept that genetic material can be introduced into disabled cells to attenuate or correct expression of a disease[3,4]. It was thought that viruses could be used as vectors and that the ability of oncoretroviral viruses to integrate into the cell genome could afford stable incorporation of a therapeutic transgene. Accordingly, replication-defective oncoretroviruses were engineered and packaging cell lines were developed for production of the retroviral vectors. Twenty years ago, Blaese and colleagues decided to take advantage of the presence of T cells in the blood of ERT-treated patients with ADA deficiency; the researchers collected the cells by apheresis and transduced them *ex vivo* with a modified oncoretrovirus containing a copy of *ADA*. Billions of T cells were collected, transduced and reinjected into two patients. Although this procedure did not correct the immunodeficiency or enable withdrawal of ERT, this very first attempt showed that the procedure was feasible and safe and led to the persistence of transduced T cells as much as 15–20 years later. This 'gene-marking' experiment confirmed the very long lifespan of mature T cells. In the late 1980s, several groups reasoned that through the use of the similar retroviral vectors, it should be feasible to achieve integration of *ADA* into the genome of HSCs and thus correct ADA-deficient SCID in a sustainable way[6]. Preclinical data obtained *in vitro* and in xenogeneic graft models looked promising. However, attempts to treat patients with ADA-deficient SCID by *ex vivo* transfer of *ADA* into either cord blood progenitors collected at birth or bone marrow progenitor cells failed, as ERT could not be interrupted in any of the patients[7,8]. This situation was at least in part the result of the fairly inefficient technology used at the time to transduce progenitor cells.

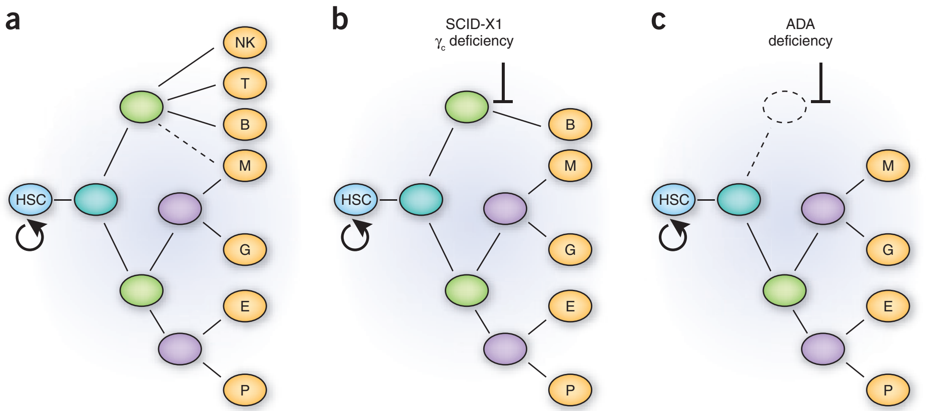

Figure 1 Block in lymphocyte differentiation resulting from a deficiency in γ_c (SCID-X1) and ADA. (**a**) Normal hematopoiesis. (**b**) Deficiency in γ_c results in impairment (⊥) in the generation of NK cells and T cells, whereas B lymphocyte differentiation is spared. (**c**) Deficiency in ADA results in premature cell death of either uncommitted lymphoid progenitors or committed progeny cells (⊥). HSC ↺ indicates HSCs with self-renewal capacity; dotted lines indicate the possible contribution of 'lymphocyte' progenitors to monocyte or dendritic cell differentiation. NK, T and B, NK, T and B lymphocytes; M, monocytes and dendritic cells; G, granulocytes; E, erythrocytes; P, platelets.

The rationale for gene therapy for SCID-X1

Meanwhile, researchers had deciphered the molecular mechanisms of other SCID conditions, including SCID-X1 (which accounts for 40 to 50% of all cases). This disease is characterized by faulty development of T cells and NK cells due to various mutations in the gene encoding interleukin 2 receptor-γ (*IL2RG*) and thus impaired expression and/or function of the γ_c cytokine receptor subunit. This finding helped researchers to understand the respective roles of IL-7 and IL-15 in triggering survival, proliferation and differentiation signals in T cell and NK cell precursors[1] (**Fig. 1**). Our research group found that a patient with SCID-X1 who had a somatic, revertant mutation of *IL2RG* had a much milder immunodeficiency and presented with detectable T cells in the blood. A detailed analysis of the repertoire of T cell antigen receptor β-chain variable segments showed that patients had about 1% of the normal T cell repertoire and prompted us to postulate that IL-7 signaling triggers enormous proliferation of a single T cell progenitor before the progeny cells undergo T cell antigen receptor rearrangements[9]. This form of 'natural gene therapy' led us to wonder whether engineered gene therapy of a few lymphoid progenitors might be sufficient to fully restore the T cell arm of the immune system, based on the selective growth advantage conferred by γ_c expression.

Luckily, several groups made substantial improvements in retroviral gene transfer technology at around this time (notably through the use of the appropriate cytokines for inducing progenitor cell cycling and fibronectin fragments to improve virus-cell contact)[4]. After years of preclinical testing, this progress enabled the initiation of technologically modern clinical trials for SCID-X1 and then ADA deficiency. For the latter disease, it was reasoned that treatment should be done in the absence of ERT to maintain the transduced progenitor cells' growth advantage.

In all, 20 patients with classic SCID-X1 disease and 27 with ADA deficiency underwent *ex vivo* gene therapy with γ-retroviral vectors in the period of 1999–2009. Five different trials were done in Paris, London, Milan and Los Angeles and at the National Institutes of Health[10–14]. The wealth of information collected is shaping the future of gene therapy. Two particular issues, genotoxicity and efficacy, merit discussion.

Genotoxicity

Five of the twenty patients that received *ex vivo*–transduced autologous CD34$^+$ hematopoietic progenitor cells for the treatment of SCID-X1 (**Fig. 2**) developed a T cell leukemic disease between 23 and 68 months later. The disease was fatal for one patient and was cured in the four others. These observations were widely commented on and were sometimes used as a justification that gene therapy (at least in its present state of development) should not be done[15–24]. All five cases featured uncontrolled clonal proliferation of T cells caused by deregulated expression of oncogenes—notably *LMO2*—as a result of integration of the vector provirus in the gene region. The enhancer activity of the viral long terminal repeat (used to drive therapeutic transgene expression) had transactivated the oncogene[25]. These results prompted in-depth analysis of retroviral insertion sites, an analysis made possible by knowledge of the human genome sequence and the availability of adequate ligation-mediated PCR technologies. It was recognized that γ-retroviral vectors insert in a semi-random manner and have a greater tendency to target gene loci (including both promoter and gene regions) with a frequency that is higher for actively transcribed genes, as is the case for a number of proto-oncogenes (including *LMO2*) in hematopoietic progenitor cells[26,27]. However, it is notable that although five of the twenty patients with γ_c deficiency (median follow-up period, 7.7 years; range, 2.5–11 years) had a serious adverse event, none of the nineteen patients with ADA deficiency that were successfully treated (median follow-up period, 3 years; range, 0.5–9 years) showed any adverse effects. This observation suggests that as-yet-unidentified disease-related factors are also involved. The occurrence of genotoxicity halted the trials and led to considerable efforts to assess and then minimize risk. On the basis of preclinical data, γ-retroviral or lentiviral vectors in which the long terminal repeat enhancer element has been removed and an internal promoter has been added represent a substantial step toward safer gene therapy, although this advance has yet to be demonstrated *in vivo*. Further improvements are expected from the development of new technology designed to achieve integration into a neutral region of the genome ('safe harbor') or gene replacement, both based on homologous recombination with engineered nucleases[28].

Efficacy

At this time, 18 of 20 treated patients with SCID-X1 and all 27 treated patients with ADA deficiency are alive. The immunodeficiency has been corrected in 17 patients with SCID-X1 (85%) and 19 patients with ADA deficiency (70%; **Fig. 2**). We now have 11 years of follow-up for the first treated patients with SCID-X1. In most patients with SCID-X1, correction of the T cell immunodeficiency is nearly complete (as demonstrated by cell-subset distribution and cell repertoire and functions), even in four of the five patients who underwent antileukemia chemotherapy. Although the overall correction of the immunodeficiency is not as complete in ADA deficiency—probably as a consequence of additional, extra-hematopoietic consequences of this condition—it was sufficient to obviate the initiation or resumption of ERT in these 19 patients. Thus, 36 patients are now enjoying a normal life and are able to

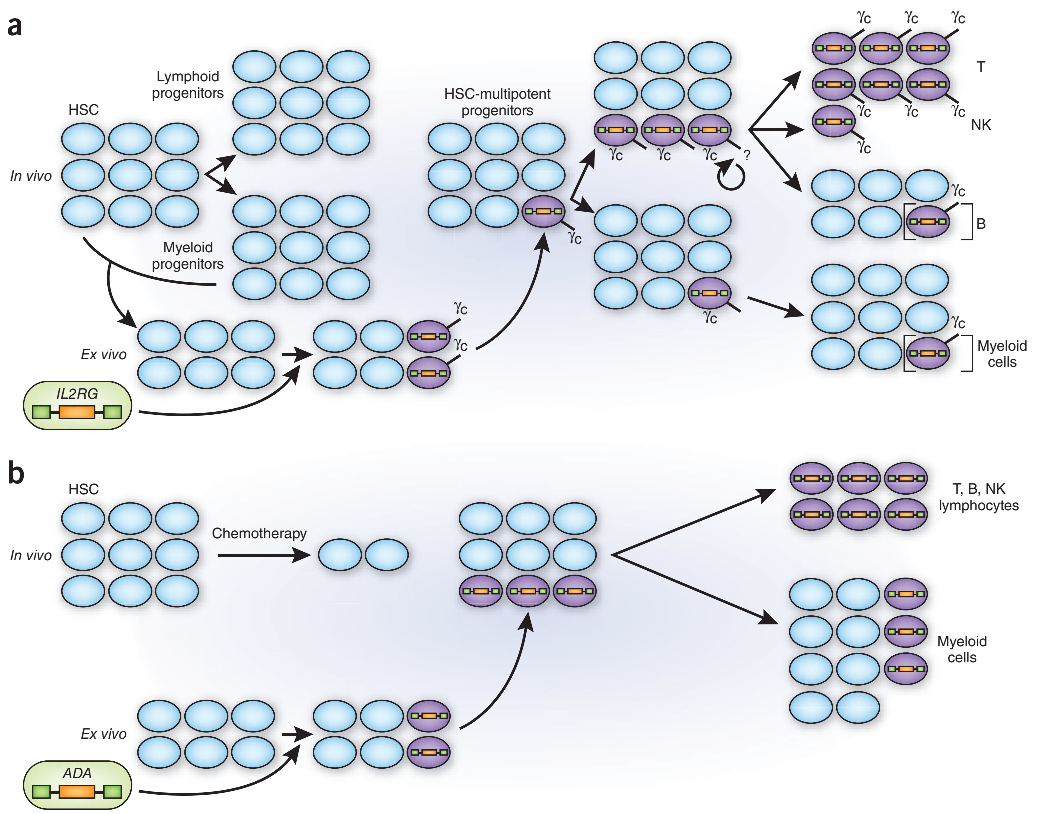

Figure 2 Gene therapy for SCID-X1 and ADA deficiency. (**a**) In SCID-X1, a combined set of CD34$^+$ HSC, lymphoid and myeloid progenitors are transduced *ex vivo* with a retroviral vector containing *IL2RG*. Once reinjected, the cells contribute to a very small fraction of hematopoiesis (red). Nevertheless, they provide precursors for persistent lymphopoiesis of T cells (and, to a lesser extent, NK cells). In contrast, most B cell differentiation and myelopoiesis originates from untransduced stem cells. Given that transduced B cells and myeloid cells are no longer detectable (brackets) in the long term, it is postulated that self-renewing T cell progenitors (↻) may persist. (**b**) In ADA deficiency, a combined set of CD34$^+$ HSC, lymphoid and myeloid progenitors are transduced *ex vivo* with a retroviral vector containing *ADA* cDNA (CD34$^+$ cells). Meanwhile, the *in vivo* stem cell pool is diminished by myeloablative (busulfan) chemotherapy so that after injection of transduced cells, a fraction of the HSCs are transduced. This enables the persistent differentiation of T cells, B cells and NK cells and detection of transduced myeloid cells (red). □▬□ indicates provirus integration.

cope with infections and do not require any therapy other than immunoglobulin substitution in some cases. This provides strong proof of principle that gene transfer can indeed result in gene therapy. These results merit comments. A genetic analysis showed that a limited number of insertion sites (around 1×10^3) are found in polyclonal T cells. This finding indicates that the T cell compartment was indeed generated from a limited number of transduced progenitors and provides a formal demonstration of the selective advantage concept on which gene therapy for SCID was based. What is unclear is whether or not this oligoclonality merely reflects the physiology of T cell lymphopoiesis. Gene therapy may serendipitously provide a surrogate way (via 'gene marking') of studying the *in vivo* characteristics of lymphopoiesis and the dynamics of lymphocyte populations in humans that may possibly also apply to healthy people.

There is still debate about whether vector-integration localization into the genome is neutral or could provide a selective advantage to some clones to proliferate and differentiate. Although both scenarios could coexist, assessment of the pattern of gene integration into polyclonal T cells tends to challenge the idea of selection in the long-term homeostasis of the T cell compartment. A remarkable observation made in one SCID-X1 trial was the variability of the longitudinally detected oncoretroviral virus-integration sites[29]. Although this result could still reflect bias in insertion detection, it could be interpreted as the successive involvement of distinct lymphoid progenitors in the generation of T cells. There is certainly a need for further studies of T lymphocyte dynamics. In the ADA-deficiency trials, it was reasoned that, given the ubiquitous deficiency, myeloablation therapy should be used to increase the ratio of transduced stem cells to untransduced stem cells (**Fig. 2a**). This protocol led to the sustained detection of not only transduced T cells but also NK cells and B lymphocytes and around 10% of myeloid cells. Detection of the very same oncoretroviral virus-integration sites in the different cell lineages demonstrated that multipotent progenitors had been transduced. No myeloablation was done in the SCID-X1 trials. Interestingly, several years after therapy, transduced myeloid cells could no longer be detected, whereas naive T cells were still present (even in patients who had received antileukemia chemotherapy in the meantime). These findings strongly suggest that ongoing thymopoiesis could originate from cells with persistent self-renewal capacity—perhaps cells located in the thymus. Finally, in the SCID-X1 trials, it was observed that reconstitution was far poorer for the NK cell lineage than for T cells. Indeed, patients do not achieve more than 10% of the control NK cell counts at 1 year after gene therapy. It thus seems likely that γ_c (IL-15)-dependent NK cell precursor proliferation and/or NK cell survival are/is lower than that of T cells, an illustration of another biological question raised by observations made in gene therapy trials.

Concluding remarks

The overall impressive efficacy of gene therapy for SCID compared with transplantation of non-HLA-identical HSCs (in which the occurrence of graft-versus-host disease–mediated serious adverse events remains a life-threatening hurdle) provides a clear rationale for continuing this therapeutic approach.

The transduction technology has now been considerably improved and safer vectors have been designed (as described above), so it makes sense to view gene therapy as an option for the treatment of SCID. Extension to the treatment of other SCID diseases (for example, deficiency in recombination-activating gene 1 or

2, Artemis, or the kinase Jak3) is being considered, based again on the anticipated selective advantage of transgene-expressing lymphoid progenitors. Further extension to other T cell immunodeficiencies or more complex immunodeficiencies (Wiskott-Aldrich syndrome, for example) is also underway, despite a smaller expected selective advantage. The combined use of lentiviral (human immunodeficiency virus–based) vectors (for more efficient integration into stem cells) and myeloablation (to increase the ratio of transduced HSC pools to untransduced HSC pools) will probably work, as observed in the HSC-based gene therapy of adrenoleukodystrophy, a disease in which no such selective advantage of transduced cells is present[30]. Just as transplantation of HSCs emerged from the pioneering work in primary immunodeficiency diseases more than 40 years ago, it is possible that the early success of gene therapy for SCID could pave the way for the development of gene-based treatments of other diseases of the hematopoietic system. The field's highs and lows have been artificially amplified by the media and the scientific community. A more cautious, better balanced view should prevail. Immunologists should also consider gene therapy for its 'bystander' effects; that is, the provision of tools for the *in vivo* study of hematopoiesis-lymphopoiesis in humans.

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The authors declare no competing financial interests.

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